Guidance for Industry and/or for FDA Reviewers/Staff

Premarket Approval Applications for Assays Pertaining to Hepatitis C Viruses (HCV) that Are Indicated for Diagnosis or Monitoring of HCV Infection or Associated Disease

Draft Guidance – Not for Implementation

This guidance document is being distributed for comment purposes only.
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Center for Devices and Radiological Health

Microbiology Branch
Division of Clinical Laboratory Devices
Office of Device Evaluation

Preface

Public Comment:

For 90 days following the date of publication in the Federal Register of the notice announcing the availability of this guidance (end date January 6, 2000), comments and suggestions regarding this document should be submitted to the Docket No. assigned to that notice, Dockets Management Branch, Division of Management Systems and Policy, Office of HumanResources and Management Services, Food and Drug Administration, 5630 Fishers Lane, Room 1061, (HFA-305), Rockville, MD 20852.

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Guidance[1] on Premarket Approval Applications (PMAs) for Assays Pertaining to Hepatitis C Viruses (HCV) that Are Indicated for Diagnosis or Monitoring of HCV Infection or Associated Disease

I. Introduction

This guidance document represents current FDA thinking regarding PMAs for IVDs that pertain to HCV infection. Such IVDs typically employ immunoassay technology for detecting antibodies to HCV (anti-HCV) or hybridization technology for detecting HCV RNA. The information in this document is based on current science, clinical experience, FDA review experience, and changes resulting from reengineering and the FDA Modernization Act of 1997 (FDAMA 97) {ref. 1}. As advances are made in science and technology, and as additional changes resulting from implementation of legislation occur, this document will be re-evaluated and revised as appropriate.

Purpose

This document provides general guidance about information FDA considers to approve class III IVDs intended for use as aids in diagnosis, prognosis, and monitoring of HCV infection or HCV-associated disease, including hepatitis C. This guidance supplements 21 CFR, Parts 800-1299. Please refer to 21 CFR, Part 814 for specific information on PMAs.

Definition

This type of device is generically intended for clinical laboratories to use for detecting or quantifying HCV antibodies, antigens, or RNA in clinical specimens.

Regulatory jurisdiction

This document does not provide guidance for licensing HCV IVDs for screening or managing donors of blood, plasma, tissue, or organs ("safety of blood and blood products"). Licenses pertaining to safety of blood and blood products are obtained from the FDA Center for Biologics Evaluation and Research (CBER), which should be directly contacted for information about preparing applications for licensure. When an HCV IVD is indicated only for safety of blood and blood products, a pre-license application (PLA) should not be submitted to CDRH. HCV IVDs for any other indication, usually diagnosis or monitoring, are regulated by CDRH.

[1] This document is intended to provide guidance. It represents the Agency's current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

II. Background

[Note: this section will be edited, with corrections, updates, and appropriate literature references, for a subsequent draft. It will also contain a paragraph on extrahepatic HCV-associated disease. Editing suggestions are welcomed.]

HCVs are one of five virus types (A, B, C, D, and E) that together account for the majority of viral hepatitis cases. Nearly 4 million Americans are infected with HCV. HCV infection is more common in minority populations (3.2% of African-Americans and 2.1% of Mexican-Americans) than in non-Hispanic whites (1.5%) {ref. 2,3}. Currently, approximately 30,000 new acute infections are estimated to occur each year. It has been estimated that HCV is responsible for 8,000-10,000 deaths annually and, without effective intervention, that number is postulated to triple during the next 10-20 years. Hepatitis C is now the leading reason for liver transplantation in the USA.

HCV is transmitted primarily by the parenteral route. Sources of infection include injection drug use, needle-stick accidents, and transfusions of blood or blood products. Because HCV is not easily cleared by the host's immunologic defenses, a persistent infection develops in as many as 85% of acutely infected individuals. This inability to clear the virus by the infected host leads to the development of chronic liver disease. Lastly, in contrast to hepatitis types A and B, there is no effective vaccine to prevent acquisition of HCV infection.

HCV is a positive-strand RNA virus, classified in the family *Flaviviridae*. An infected individual circulates a population of closely related, but heterogeneous, viral genomes (a quasispecies). Comparison of nucleotide sequences representing different HCVs enables classification of HCVs into several genotypes and subtypes {ref. 4}. Data on the natural history of hepatitis C are limited, because the onset of infection is often unrecognized and the early course of the disease is indolent in many individuals. The natural history of this disease may differ according to geography, alcohol use, HCV characteristics (e.g., genotype, load, and quasispecies complexity), coinfection with other viruses, and other unexplained factors.

Viremia can be detected, as HCV RNA in serum or plasma, within 1-3 weeks after exposure. Within 50 days (mean; range, 15-150 days), virtually all individuals develop liver cell injury, as manifested by elevated serum concentration of alanine aminotransferase (ALT). The majority of individuals are asymptomatic and anicteric. Only 24-35% develop acute illness: malaise, weakness, or anorexia; fewer develop jaundice. Fulminant liver failure following HCV infection has been reported, but rarely. Anti-HCV becomes detectable during the course of illness. Anti-HCV can be detected in 50-70% of patients at onset of symptoms and in approximately 90% of patients 3 months after onset of infection. HCV infection appears to be self-limited in only 15% of infections. Apparent recovery is characterized by disappearance of HCV RNA from blood and return of ALT and other "liver enzyme" concentrations to normal.

Approximately 85% of HCV-infected individuals fail to clear the virus within 6 months and develop chronic infection with persistent, sometimes intermittent, viremia. Most also develop chronic hepatitis, with histopathologic changes in the liver. This capacity to produce chronic hepatitis is one of the most striking features of HCV infection. The majority of individuals with

chronic infection have elevated ALT levels that can fluctuate widely. About one-third of chronically infected individuals have ALT levels that are persistently within reference ranges. Circulating anti-HCV and HCV RNA can be detected in virtually all actively infected individuals. Chronic hepatitis C typically is an insidious process, progressing in most individuals at a slow rate and without symptoms or physical signs during the first two decades after infection. A small proportion of patients (<20%) develop nonspecific symptoms, including mild intermittent fatigue and malaise. Although many HCV-infected individuals with normal ALT levels have been referred to as "healthy" HCV carriers, their liver tissue has had histopathologic evidence of chronic hepatitis. In many patients, symptoms first appear as advanced liver disease develops. The rate of progression is highly variable. Long-term studies suggest that most patients who develop cirrhosis have ALT elevations (that may be intermittent). There is an inconsistent relationship between ALT levels and severity of histopathologic changes.

Chronic HCV infection leads to cirrhosis in at least 20% of individuals within 2 decades after infection. Occasionally, cirrhosis and end-stage liver disease may develop rapidly, especially among patients with concomitant alcohol ingestion. Chronic HCV infection is also associated with an increased risk of hepatocellular carcinoma (HCC). The prevailing concept is that HCC develops, over the course of approximately 30 years or longer, within a chronic hepatitis-associated background of inflammation, regeneration, and fibrosis. Most cases of HCV-associated HCC occur in the presence of cirrhosis.

A variety of laboratory methods are available for diagnosis or management of HCV infection and HCV-associated diseases. Tests that detect anti-HCV include enzyme immunoassays (EIAs), which typically contain several HCV antigens (encoded by the putative core and nonstructural genes and produced via recombinant DNA technology), combined on a solid phase. Strip immunoassays (SIAs) are typically used for supplemental, or second-step, detection of anti-HCV in EIA-positive specimens; while SIAs use the same or similar HCV antigens as EIAs, these antigens are separated on a membrane strip so that antibodies to each can be detected in a manner similar to immunoblotting. In addition, several hybridization-based assays have been developed for detecting HCV RNA in serum or plasma. These HCV RNA assays, none of which are currently approved or licensed by FDA, use target amplification methods such as polymerase chain reaction (PCR) or direct hybridization techniques such as branched DNA (bDNA). Liver biopsy can be performed to estimate the extent of hepatic injury, inflammation, or fibrosis. Although some histopathologic findings are characteristic of HCV infection, such as portal lymphoid aggregates, steatosis, and bile duct injury, these alone are not sufficiently specific to establish a diagnosis of hepatitis C. There are not any standardized, readily available tests for detecting HCV antigens in liver or blood.

III. Clinical Significance and Utility

The submission should include the following information about the assay:

A. Description of antibodies, antigen, or nucleic acid detected.

B. Description of disease or syndromes associated with infection caused by HCV.

C. Description of epidemiology, including prevalence and groups at risk for infection and disease.

D. Discussion of historical and currently accepted methods used to detect HCV and HCV infections, including any approaches for detecting HCV antibodies, antigen, or RNA.

E. Comparison between the new assay and any previously licensed or approved devices (i.e., similarities and differences)

F. Description of reference ("gold standard") methods, if available, for detecting evidence of HCV in clinical specimens.

G. Discussion of genetic variants of HCV, their proposed clinical significance, and their known or potential impact on the new assay.

H. Discussion of the significance and clinical implications of false-positive and false-negative results.

IV. Device Description

For a PMA that pertains to an IVD for HCV infection, key attributes include intended use (antibody, antigen or nucleic acid detected) of the assay, technology for detection or quantitation, types of specimens to be tested, and clinical indications for use. PMAs should include information for adequately characterizing the new assay when it is used with clinical specimens, including:

A. Intended Uses

should be described, and the following information should be provided:

1. Analyte(s) detected, qualitatively or quantitatively.
2. Types of specimens (matrices) that are intended for testing.

B. Contraindications

should include use in testing or managing donors of blood, plasma, tissue, or organs (unless licensed for such uses by CBER). Please refer to *Screening or managing donors of blood, plasma, tissue, or organs* (IV.C.2, below).

THIS CONTRAINDICATION SHOULD BE DISPLAYED ON PACKAGE LABELS AND THE PACKAGE INSERT IN WAYS TO MINIMIZE THE POSSIBILITY THAT THE ASSAY COULD BE USED, INADVERTENTLY OR INTENTIONALLY, FOR SUCH DONOR INDICATIONS. Suggestions include: distinctive type or shape of kit boxes and reagent vials, distinctive color or font for printed materials, and a prominent contraindication-statement on the assay box and in the package insert (please see section V.A.2.c below on analytical sensitivity as it pertains to this contraindication).

C. Indications for Use

should be addressed by considering the population(s) for which the assay is intended and its clinical utility. Examples of specific indications include:

1. Evidence of HCV infection, not specified with regard to state of infection or associated disease.

Assays for anti-HCV should demonstrate performance for at least this indication. This indication is not appropriate for assays that detect HCV antigens or RNA.

FDA recognizes that the vast majority of infected Americans are chronically infected, with or without hepatitis, and that the predominant use of anti-HCV assays will be for initially identifying such infected individuals. Studies that determine performance for this indication (see below, V. B and C.1) will predominantly be based on data from comparative-assay testing of sera for anti-HCV (Table 1) but without necessarily having detailed information about the state of HCV infection or disease in the individuals from whom the sera were collected. These sera are also highly likely to represent chronically infected individuals. Therefore, it is thought that specimens can be studied to determine safety and effectiveness with regard to the major indication for anti-HCV testing (i.e., initial identification of individuals who are presumed to be chronically infected), even without extensive characterization of the individuals who supply specimens for clinical studies. The limitation to using this as a sole indication for approval is the lack of demonstrated performance with regard to precise states of HCV infection, particularly acute HCV infection or acute hepatitis C. For the latter, understanding of clinical sensitivity and specificity are crucial for making accurate diagnoses but such infections occurred infrequently at the time (1998-99) this document was prepared. When FDA consulted with several experts on hepatitis C, the consensus was that public health would be well served by having this indication, in spite of its limitation.

If this is the only indication for which performance has been demonstrated, the Intended Use section in the package insert would contain the following or similar text:

"[Assay name] is an enzyme immunoassay for qualitative detection of antibodies to HCV as evidence of HCV infection that does not specify the state of infection or associated disease. A positive result does not discriminate between active or inactive (resolved) infection or among types of active infection (acute or chronic, asymptomatic or symptomatic, with or without hepatitis). A negative result does not rule out acute infection. Performance has not been established for aiding diagnosis of acute hepatitis C, chronic hepatitis C, or other HCV-associated diseases, or for evidence of recovery from HCV infection."

2. Screening or managing donors of blood, plasma, tissue, or organs.
Such use requires a license from CBER, which should be directly contacted about preparing applications for licensure.

3. Aid in detecting asymptomatic acute infection with HCV
(e.g., asymptomatic following exposure). Please note that "infection" refers to active HCV replication but not necessarily to HCV-associated disease.

4. Aid in detecting asymptomatic chronic HCV infection.
This is likely to become a major indication as DHHS recommendations for testing of many at-risk Americans {ref. 3} become manifest.

5. Aid in diagnosis of acute hepatitis C
(acute infection with symptoms or biochemical evidence of hepatitis)

6. Aiding diagnosis of chronic hepatitis C

7. Aiding diagnosis of hepatitis C (indiscriminate between acute and chronic).
Like Indication 1, patients studied would predominantly represent chronic hepatitis C and the package insert's Intended Use statement would contain a disclaimer, such as:

"A positive result does not discriminate between acute or chronic hepatitis C. A negative result does not rule out acute infection. Performance has not been established for aiding diagnosis of, specifically, acute or chronic hepatitis C or for evidence of recovery from HCV infection."

8. Monitoring HCV infection
includes at least several important indications:

a. Prognosis of acute HCV infection
b. Prognosis of chronic HCV infection without antiviral therapy
c. Predicting response of chronic HCV infection to antiviral therapy
d. Monitoring response of chronic HCV infection to antiviral therapy
e. Prognosis of chronic HCV infection after antiviral therapy is completed or discontinued

Although all of these indications are plausible, current peer-reviewed scientific literature primarily discusses Indications 8.c. and 8.d. This list could grow if treatment becomes available for acute infection. These indications are almost exclusively for assays that qualitatively or quantitatively detect HCV RNA. (There are reports that anti-HCV assays can be used to monitor recovery from HCV infection, with or without antiviral therapy; this indication has not been addressed in this document).

9. Novel indications for use
can be considered or assessed, in which case the manufacturer should recognize current standards of diagnosis and care, as expressed in the peer-reviewed scientific literature, and communicate with FDA as early as possible.

D. Detailed Principle of Device Methodology
The submission should include thorough explanations of all aspects of the test method, including a complete description of the following items as appropriate:

1. Methodologic principles, with a brief history of the specific technology upon which the device is based.
2. Specimen processing.
3. Controls and calibrators, that are provided, and the functions of controls (i.e., what are they controlling for and how?). Whether provided with the assay or recommended via package-insert instructions, the manufacturer should specify at least one negative control and one positive control that duplicate or simulate clinical specimen types (matrices) that would be tested; i.e., controls for the complete assay. When controls are not provided, package-insert recommendations should be

supported by data to ensure that assay users will be consistently able to produce, store, and process the control materials so as to yield reproducibly valid results.

a. Qualitative assays should have at least two types of controls: negative, and a "low" positive, yielding a value close to the assay cutoff.

b. Quantitative assays should have at least two calibrators at appropriate points within the clinically relevant range of the assay.

c. Calibrators for quantifying HCV RNA should be characterized in terms of reference material, quantified by one or more independent methods. Units for HCV-RNA calibrators should be appropriately designated; e.g., "copies" or "genomes" should not be used unless the number of molecules has been determined. In many cases, and until there are well accepted standard reference materials for HCV RNA, it will be more appropriate to create an arbitrary but technologically accurate designation (e.g., "ABC Co. HCV RNA signal-generating units") with an explanation of its microbiologic or clinical relevance.

d. Matrices for controls and specimens should be identical. If a specimen-identical control matrix is not used, a rationale and data to support use of an alternative control matrix should be provided.

e. Controls for inhibition (internal or external, when appropriate).

4. Other reagents provided in the kit or recommended for use; their functions in the assay.

5. Cutoff value(s) or reporting threshold, for qualitative assays.

6. Limit of detection and limit of quantitation, for quantitative assays.

7. Safety aspects for performing the assay.

8. Software elements and dedicated instrumentation, including:

   a. Algorithms used to calculate results in either dedicated or non-dedicated instruments.

   b. Mathematical curve-fitting methods(s) used to calculate results via instrument-related software.

9. Materials required but not provided.

E. Specimen collection, transport, and processing materials
(that are included in the kit, specified, or recommended) – the manufacturer should specify details pertaining to optimal and unacceptable procedures for specimens that would be tested with the IVD:

1. Procedures to assure that specimens are appropriately collected, transported, and processed

2. Types and applicable volumes of all appropriate specimens for testing and the effects of testing inadequate or inappropriate specimens

3. Appropriate processing and transport conditions (e.g., time and temperature) for each type of specimen and the effect(s) of inappropriate processing and transport

4. Recommended storage time and temperature and the effect(s) of inappropriate storage time and temperature.

V. Performance Characteristics

To enable marketing of IVDs, FDA requests certain types and amounts of data from preclinical (analytical) and clinical studies. Submitted data should reflect intended use, indications for use, and technological characteristics of the IVD. These data should be analyzed by using appropriate statistical methods. Such data and analyses should be sufficient to enable determination of the assay's safety and effectiveness.

Complete protocols, including all laboratory procedures, should be provided for all studies. All testing to establish performance characteristics should be performed by using the "finished product" design (i.e., that which would be marketed), according to instructions in proposed product labeling. Raw laboratory data ("line data") with quality control results should be submitted in print (hard copy) and disk form. Data should be presented with analyses and conclusions. Explanations should be included for unexpected results and any deviations from protocols, such as additional testing. In addition, appropriate tables and graphs (e.g., scatter plots, histograms, and receiver-operator curves [ROC]) are encouraged for summarizing or clarifying analyses and conclusions.

Any clear format is acceptable for sections of the PMA that pertain to performance characteristics. From past experience, FDA has several suggestions for facilitating review:

- include a reference volume that contains a detailed table of contents for the entire PMA, a short "executive" summary of the PMA, a copy of the proposed package insert and other labeling, and, if possible, an index for the PMA (i.e., extensive cross-referencing is very helpful)
- use the format found in most scientific manuscripts; i.e., Abstract, Introduction, Materials and Methods (study protocols, with package inserts for comparative assays in appendices), Results and Conclusions (data and analyses for all studies, with line data in appendices), Discussion (optional), and References
- insert each table and graph within the appropriate text section, rather than placing them at the end of text.

All assays should have at least one cutoff to distinguish between types of results. It may be appropriate to have different cutoffs for different indications for use. During preclinical studies, tentative cutoff(s) should be set by using any of several valid approaches; examples include:

- a number representing the mean value plus several standard deviations for specimens known not to contain the detected or measured analyte
- a number between values for specimens known to contain the analyte and values for analyte-negative specimens
- ROC analysis.

Later, clinical studies should validate the cutoff(s) for each indication for use by testing well-characterized specimens or specimens from well-characterized individuals. FDA recognizes that it may be necessary to make minor cutoff changes during or after clinical studies. Any cutoff changes, however:

- should be justified (from analysis and post-change re-analysis of data)
- may need to be tested in subsequent clinical or reproducibility studies.

A. Preclinical (Analytical) Laboratory Studies

Testing should be done in-house or at a designated laboratory facility as part of assay development phases. The following types of studies should be performed to determine operational parameters and assess performance:

1. Setting cutoff(s) or calibration curve

a. For any assay:
   (i) Evaluate specimens containing analyte (e.g., from patients with hepatitis C or other manifestations of HCV infection) and specimens known or thought not to contain analyte (e.g., from asymptomatic, healthy individuals or from patients with other forms of hepatitis).
   (ii) Describe the rationale for setting assay cutoff(s). Furnish descriptive information and data to show how each cutoff distinguishes between "positive" and "negative" results.

b. For a qualitative assay, FDA strongly recommends that the manufacturer describe the basis for and then establish a least one equivocal (gray) zone; different equivocal zones might be appropriate for different indications for use.

   NB: Traditional microtiter-plate EIAs for anti-HCV essentially designate all values above a cutoff as equivocal; i.e., specimens that yield "initial" positive results are retested in duplicate before reportable results are interpreted. At this time, ALL ASSAYS INDICATED FOR SAFETY OF BLOOD OR BLOOD PRODUCTS SHOULD BE DESIGNED TO ADHERE TO SUCH A REPEAT-TESTING ALGORITHM. Manufacturers should contact CBER for specific and updated recommendations. While there is no requirement for such an algorithm when an assay would be used for diagnostic or monitoring indications, a different type of testing and interpretation algorithm should be extensively supported by data and analysis from the manufacturer.

c. For a quantitative or semi-quantitative assay**,** the manufacturer should describe the rationale for determining lower and upper limits of detection (dynamic range) and for clinically relevant range(s) of quantitation or semi-quantitation.

2. Analytical sensitivity

should be determined for:

a. Each of the specimen matrices and diluents that would be used.

b. Strains representing diversity (e.g., geographic, genotypic, or phenotypic / drug resistance) among HCVs, including all variants for which manufacturer wants to make claims. Such data should include results of searching Genbank (National Center for Biotechnology Information,

http://www.ncbi.nlm.nih.gov) or other comprehensive databases for identity between sequences represented by the assay's analyte-specific reagents and those of strains or genotypes for which the manufacturer wants to make claims.

c. For anti-HCV assays, the manufacturer's goal should be to achieve analytical sensitivity equal to or higher than that of the latest "generation" of EIAs licensed for use on donors of blood or blood products (during early 1999, such assays represented a third "generation"). If such sensitivity cannot be achieved or if it is not appropriate for certain indications for use, package labeling for the assay, if approved, should clearly indicate lower sensitivity.

ASSAYS THAT HAVE NOT BEEN LICENSED BY CBER FOR BLOOD-PRODUCT INDICATIONS SHOULD BE CLEARLY LABELED AS CONTRAINDICATED FOR SUCH INDICATIONS. FOR EXAMPLE, THE OUTSIDE OF THE KIT SHOULD INDICATE THIS CONTRAINDICATION IN BOLD LETTERS THAT CONTRAST WITH OTHER TEXT (Please see above, IV.B). THE MANUFACTURER SHOULD USE A DIFFERENT COLOR FOR LABELING (KIT, VIALS, PACKAGE INSERT, ETC) THAN THOSE FOR OTHER ASSAYS IT DISTRIBUTES.

Several possible approaches to determining analytical sensitivity include:

- end-point dilution
- earliest detectable reactivity in groups of serially collected samples ("seroconversion panels"), where each group represents an individual
- comparison to (standard) reference materials
- comparing results for analyte-positive specimen with analyte concentration determined by one or more independent methods
- for assays that detect HCV antigen or RNA, establishing limits of detection (LOD) or endpoints by determining the minimum detectable number of analyte molecules and, if possible, a minimum number of 50% chimpanzee (or, if available, cell-culture) infectious doses of HCV.

3. Limits of Quantitation (LOQ) for quantitative and semi-quantitative assays

a. Dynamic range should be established, by determining lower limit (analytical cutoff) and upper limit for quantifying the concentration of HCV antigen or RNA or for semi-quantitation of anti-HCV.

b. Arithmetically or geometrically linear performance and nonlinear performance should be determined (data points should be presented with an expression of their variability, such as 95% confidence intervals).

c. Quantitative accuracy and precision throughout the dynamic range should be determined.

d. Approximate interpretations should be established for results that represent different concentrations of analyte (analogous to setting cutoffs: please refer to section IV.A.1, above).

4. Specificity for detecting HCV RNA
should be determined by:

a. Searching Genbank or other comprehensive nucleic-acid databases for similarity between sequences of the assay's analyte-specific reagents and those of other entities.

b. Performing nucleic-acid detection studies on well-characterized isolates and strains of microorganisms (e.g., ATCC or WHO reference strains):

(i) These microorganisms should represent:
- closely "related" flaviviruses, if any are identified
- unrelated genera that cause hepatitis and other HCV-associated syndromes
- other flora (pathogenic or commensal) found in assayed matrices.

(ii) The PMA should contain:
- lists of genus, species, applicable reference numbers, sources, and tested number for each microorganism
- documentation of inocula and of methods for identifying, characterizing, and quantifying these microorganisms.

(iii) Cross-reactivity studies should be performed by using DNA or RNA representing a large excess of these microorganisms; i.e., the concentration of tested nucleic acid should be at least 1000-fold greater than the minimum detectable concentration of HCV RNA. Use caution when concentrations of different nucleic acids are expressed in terms of the same units: unit names should be interchanged only when the determining method(s) are recognized as equivalent.

5. Interference studies
should determine if assay results are affected by potentially interfering substances (or "inhibitors") in proposed specimen types, matrices, or processing reagents. Examples of substances that may interfere with detection of anti-HCV, HCV antigen, or RNA include:

a. Endogenous substances likely to be present in specimens (e.g., triglycerides, bilirubin, hemoglobin, proteins, therapeutic drugs, or illegal drugs). For studies, the source of such endogenous substances should be actual human specimens (that will contain the range of metabolic permutations of each substance) rather than purified products.

b. Exogenous substances that may have been introduced to individual specimens or an archived collection.

c. Possible cross-reactivity with other microorganisms, which should be studied by using high concentrations of such microorganisms: please refer to section IV.A.4.b.(iii), immediately above.

6. ***Validation of methods for decontamination and specimen inactivation***
   If the manufacturer includes reagents (e.g., uracil-*N*-glycosylase, formamide, psoralen derivatives, or restriction enzymes) or recommends procedures (e.g., irradiation) for, respectively, inactivating contaminating nucleic acids or inactivating HCV, the following should be studied:

   a. Performance of the reagent or procedure.

   b. Challenge of decontamination procedures with known contaminants.

7. ***Validation of recommendations for specimen collection, transport, and storage***
   Real-time stability studies should determine optimal and permissible conditions for each proposed matrix (and each anticoagulant, if plasma would be used). These studies should evaluate effects of specimen collection, transport, and storage effects on assay results, particularly with regard to inhibition of HCV RNA detection.

8. ***Infectious agents in reagents derived from humans***
   PMAs should include data from testing, via FDA-approved methods, to determine if human-derived reagents contain infectious agents such as human immunodeficiency virus and hepatitis B virus (HBV).

9. ***Validation of reagent stability***
   Real-time studies should determine if expiration dates are accurate. Studies should also evaluate performance of any indicators that are provided for evidence of improper storage.

10. ***Reproducibility (precision)***
    Any statistically valid approach can be used to determine how well the assay yields the same result on repeated determinations. Typically, each specimen is represented in multiple aliquots (e.g., triplicate) and studies are performed on multiple assay lots at multiple sites, via multiple runs on multiple days. Other general considerations include those in NCCLS Guideline EP5-A and in the FDA Guidance Document, "Review Criteria for IVDs for Detection of IgM Antibodies to Viral Agents." In particular:

    a. Reproducibility should be studied for at least three lots of the assay.

    b. These studies should be performed in at least three sites, one of which may be in-house, for each type of laboratory setting in which the assay would be used. For example, if a single-unit qualitative anti-HCV assay would be used in clinical laboratories and in a point-of-care setting, its reproducibility should be determined in both environments.

    c. If the assay is automated, studies should be performed with at least three unique (i.e., different serial numbers) instruments.

    d. A different group, or panel, of specimens should be studied for each type of specimen matrix to be used with the assay.

e. A different group of specimens should be studied to represent (in the form of antibody, antigen, or RNA) each HCV genotype or variant that the assay is intended to detect.

f. Analyte concentrations in specimens should represent a clinically relevant range; i.e., concentrations should be those encountered in clinical practice. All such specimens should be actual clinical specimens or simulated specimens that are created by diluting analyte from a human specimen into analyte-negative human matrix. It is especially important to include at least two specimens that will yield close-to-cutoff values; i.e., both above (analyte present at very low concentration) and below the cutoff. For qualitative assays, it is often useful to include specimens that yield the cutoff value, 1.2 x the cutoff value, and 0.8 x the cutoff value. There should be a sufficient number of specimens to determine validity of the assay's equivocal zone(s).

   For quantitative assays, at least two additional specimens should be studied. These specimens should represent upper and lower thresholds for clinical decisions that pertain to each indication for use. One specimen should contain a high concentration of analyte, where "high" refers to the upper limit of clinical relevance for a particular indication or to the upper limit of the analytically-determined dynamic range, whichever is lower. The other specimen should contain a low concentration of analyte, where "low" refers to the lower limit of clinical relevance for that indication or to the lower limit of the analytically-determined dynamic range, whichever is higher.

g. Specimens for these studies should be masked; i.e., personnel who perform and interpret the data should be blinded with regard to presence or amount of analyte in each specimen.

h. Each run should be performed according to the assay's instructions for use in the clinical protocol, including provisions for quality control and calibration: these instructions should be identical to those in a draft package insert. Failed runs should be documented in the PMA.

i. Data analysis and presentation for each specimen's results should include determination of intra-assay, inter-assay, inter-lab and total variability. Analysis of Variance (ANOVA) is an example of an appropriate statistical technique for data analysis; if ANOVA were used, the manufacturer would also determine and report sums of variance, standard deviation of variance and 95% confidence intervals.

j. Data presentation for qualitative assays should also include:

   (1) For each specimen, the percent of study results that were identical to expected results

   (2) Calculation of variability, which is typically expressed as percent coefficient of variation (%CV) for numerical values of assay results.

11. Additional studies

are recommended to determine effects, if any, with regard to:

a. Prozone (high dose hook)

b. Transport or other stress on kits

12. Instrument performance

a. Rationale for instrument and software algorithms should be provided.

b. Automated systems should be studied for evidence of sample or reagent carryover.

c. Error messages – manufacturer should describe, explain, and provide data for their effectiveness.

B. Design and Protocols for Clinical Studies: General Considerations

Appropriate clinical studies should determine if the assay is safe and effective with regard to each claimed indication for use. The manufacturer of an anti-HCV assay should, at a minimum, perform studies to determine safety and effectiveness for Indication 1 (evidence of HCV infection, not specified with regard to state of infection or associated disease; please see sections on indications for use in IV.C above and V.C below).

To optimize clinical study design before studies are begun, manufacturers are encouraged to contact FDA in any manner that is helpful to them. Per Sections 513(a)(3)(D) and 520(g)(7) of FDAMA 97 {ref. 1}, manufacturers may request a formal protocol review that seeks agreement on particular items. Alternatively, protocols may be submitted for general review and comment. The PMA should include copies of all protocols. Each protocol should describe design and procedures including:

1. Intended use, indications for use, and indicated populations
The new assay should be studied to determine if the manufacturer's claims are supported.

2. Statistically based criteria for the number of individuals or specimens
that will be studied for each claimed indication; these criteria should predict how the studies will demonstrate safety and effectiveness and should estimate the uncertainty in such demonstration.

3. Study sites
Clinical studies should be performed at three or more laboratory sites; at least two should be independent (i.e., not affiliated with the manufacturer). The number of sites should be addressed by considering variables such as types of population for which the assay is intended, complexity of assay performance, familiarity with assay design, and interpretation of results. All laboratory sites should represent a setting that would be indicated for actual post-approval testing. Each study laboratory should be identified by institutional name and address and by the name, title, and phone number of the responsible investigator(s). Ideally, the protocol should be identical for each type of laboratory in which the assay will be studied. Any site-to-site variables should be explained. Investigators should

understand that strict adherence to the protocol is critical. Any deviations from protocol should be documented in the PMA and support should be provided for including data from portions of the study in which protocol deviations were made.

4. Types of studies and specimen collections

A prospective study, following a design to determine performance for a particular indication for use in a particular population, is the optimal type of study. If the specimens have been properly maintained (see below, IV.B.7) and no biases were introduced by selecting certain specimens, it does not matter that the study was performed in the past.

Prospective collections of specimens representing routine submissions to a clinical laboratory are useful but relatively undefined. The manufacturer should recognize and indicate, in the PMA, the uncertainties and biases of such a collection (if the assay is approved, the package insert should also indicate these biases). If such specimens have been submitted for detecting evidence of infection with one of the hepatitis viruses, it is presumed that the physician suspected infection so each patient represents an at-risk population. In addition, such specimens represent, via sampling, the range of received specimens for the type of laboratory that is performing the study; the protocol and PMA should describe the population(s) that the laboratory serves. However, without additional information about an individual, it would not be known if testing was ordered for (i) aiding diagnosis of an acute, chronic, or past process (and magnitude of the physician's suspicion for that diagnosis); (ii) monitoring recognized disease, with or without therapy; or (iii) pre- or post-vaccination assessment. Moreover, it would not be known if a tested individual is ill. While the information obtained from such a collection cannot be used for calculating a clinical performance parameter, such as clinical sensitivity, clinical specificity, or predictive values, it may be useful for determining one (i.e., high prevalence) type of "Expected Values."

The manufacturer should also recognize the biases inherent in specimens (archived or collected during studies) that represent selected cases of hepatitis C or other diseases. Such cases are unlikely to represent the range of specimens submitted to a laboratory, which, in turn, often represent patients with atypical laboratory results. Manufacturers should determine if, and document that, "seroconversion panels" are serially collected specimens from a single individual (caution: they may represent multiple individuals or artificial specimens created by dilution).

5. Laboratory results as inclusion, exclusion, or characterizing criteria

a) Comparative assays for evidence or characterization of HCV infection

(1) FDA-licensed or -approved assays

should be used for detecting antibody evidence of HCV infection. These assays should be selected for optimal performance with regard to

indications being studied. (The latest "generation" will usually represent the highest available analytical sensitivity and specificity.) These assays should be performed and interpreted according to package-insert instructions; any off-label deviations should be justified by presenting independent data to support such uses. Examples of such off-label uses would be "initial" (single-aliquot) testing only for a currently licensed anti-HCV EIA (package inserts specify "repeat" triple-aliquot testing) or interpreting "indeterminate" SIA results as evidence of anti-HCV. Typical interpretation of comparative-assay results for studies is shown in Table 1.

Table 1. Comparative-assay results[a] as evidence of HCV infection, per package-insert instructions: interpretation for categorizing specimens or individuals in studies

1st step (EIA) result	2nd step (SIA) result [b, d]	Interpretation → Category
Negative (initial or repeat)	Not done	**Not HCV-infected**
Positive (repeatedly reactive via testing of 3 aliquots)	Negative or Indeterminate	
Positive (repeatedly reactive)	Positive	**HCV infection, state or associated disease not determined**
Study specimen: Negative **AND** *Later specimen*: Positive (repeatedly reactive) [c]	*Study specimen:* Not done **AND** *Later specimen:* Positive [c]	**Anti-HCV seroconversion (refer to 6.b.[2])**
Study specimen: Positive **AND** *Later specimen*: Positive (Positive ≡ repeatedly reactive) [c]	*Study specimen*: Negative or Indeterminate [d] **AND** *Later specimen:* Positive [c]	
Initially reactive (testing only of single aliquot)	Not done, Negative, Indeterminate, or Positive	**None (incomplete testing) → exclude specimen [d]**
Positive (repeatedly reactive)	Not done	

[a] Historical or study-generated data, from EIA(s) and SIA(s) that should be specified in protocols; analyzed results should be those from first-time testing; i.e., specimens should not be retested to "resolve discrepant" new-assay results.

[b] 2nd step – if assays are not FDA-approved, protocol design and data should be provided to justify use in studies as a criterion for evidence of HCV infection; for recommendations on HCV-RNA assays, please refer to section V.B.6.b.(1) below.

[c] Later specimen should be collected within 3 months of study-specimen. Detection of HCV RNA in study specimen would be independent evidence of infection and establish that the infection was active when the specimen collected; please refer to section V.B.6.b.(1) on detection of HCV RNA.

[d] Alternative (off-label) testing algorithms can be used if protocol design and data support use. FDA recognizes that some medical institutions currently (1999) specify lesser criteria, especially if individuals are at high risk of HCV infection, but recommends at least two separately detected pieces of evidence (among Positive results for repeated-EIA, SIA, or HCV RNA) because of the high frequency of false-positive EIA results among low-risk individuals {ref. 2,3}.

(2) Non-approved assays

should be avoided when approved or licensed assays are available, as is the case with EIAs and SIAs for anti-HCV. However, certain indications should be supported by HCV-RNA testing, for which no assays are currently (June 1999) approved; please refer to the section V.B.6.b.(1) below, on detection of HCV RNA as a marker of active infection.

b) Other appropriate lab findings

should be documented in the line data for each individual or specimen. Results of serologic, nucleic-acid, hematologic, or biochemical testing usually provide information for additional characterization or categorization of individuals or specimens. For example, analysis of study data could be aided by evidence of infection with other viruses that could modulate replication of or immune responses to HCV.

Individuals infected with other hepatitis viruses, without evidence of HCV, constitute subgroups for implying or determining clinical specificity (for examples, see Table 3A). Protocols should include types of specimens, assays, interpretations, and categories in a manner similar to those in Table 3. These data would not, in general, be a basis for excluding an individual or specimen.

6. Inclusion and exclusion criteria for individuals

Information about individuals is highly recommended in studies to support all indications for use except, possibly, "evidence of HCV infection, not specified with regard to state of infection or associated disease" (Indication 1, in sections IV.C.1 above and V.C.1 below). Protocols should describe studies of well-characterized individuals, in which the goals are to determine performance that pertains to the indications for use that the manufacturer wants to claim. An explanation should be provided for each group of individuals that will be studied, especially if they are represented by an archive of frozen specimens. Protocols should indicate how individuals have been or will be characterized (i.e., the criteria for categorizing or excluding them) and how these criteria will be documented. For the latter, FDA recommends case report forms or an electronic database (arranged like case report forms) that will be the same for, and completed at, each clinical-study site; blank "forms" should be attached to protocols. A physician's diagnosis, without the data to support it, is not an acceptable criterion for categorizing patients.

The manufacturer should develop study algorithms that outline how individuals and results will be categorized to enable appropriate analysis of data. Figure 1 is an example of a recommended algorithm for studying performance of a new EIA for anti-HCV, indicated for "diagnosis of chronic hepatitis C" (Indication 6, in sections IV.B.6 above and V.C.6 below that pertain to Indications for Use).

a) Hepatitis

For including or excluding individuals from studies of performance for indications that pertain to diagnosis of acute or chronic hepatitis C, the manufacturer should select combinations of criteria for hepatitis among several

criteria that are recognized. FDA makes this recommendation because individual criteria are not sufficiently sensitive or specific, or may not be available, to be used as a required or only criterion. These recognized criteria include:

- Symptoms
- Physical signs
- Biochemical evidence, such as elevated serum concentrations of ALT or other markers of hepatocellular damage. The manufacturer should define "elevated" with regard to reference range(s) at the labs where the assays are performed.
- Histopathologic evidence, which should be presented in the line data by using current standard terminology; the Knodell scoring system is recommended, especially if changes in histopathologic evidence would be used as a criterion of change in disease for a monitoring indication (please see V.C.8.)

b) Active HCV infection

(1) Detection of HCV RNA

The presence of HCV RNA in blood indicates that HCV is replicating. At present, HCV RNA is the only readily detectable marker of active infection. While there are not any approved HCV-RNA assays and high variability has been recognized as a problem among current assays (home-brew and commercial) and laboratories {ref. 5}, FDA believes that it is appropriate to consider preclinical and limited clinical data for supporting use of HCV RNA detection as a criterion of active infection. Such data {ref. 6} should represent studies of:

- Analytical sensitivity and specificity, including cross-genotype reactivity. Analytical sensitivity should be characterized in terms of reference material, quantified by one or more independent methods, that can be interpreted by and is available to people outside of the labs that perform the HCV-RNA assays.
- Dynamic range, for quantitative assays
- Reproducibility
- Temporal patterns of detectability (qualitative assay) or HCV-RNA concentrations in individuals with or without FDA-approved antiviral therapy (i.e., to determine how the assay performs in serially-collected specimens that represent, respectively, situations in which HCV RNA concentrations might or might not change).

One source of such data can be peer-reviewed articles in scientific journals. For testing patient specimens during clinical studies, FDA strongly recommends use of the same assay at all study laboratories or at a single independent lab. If more than one assay is used, at different labs or because historical data is cited, the PMA should contain sufficient information to enable interpretation of results from each HCV RNA assay (e.g., data from quantified reference materials). By considering unapproved HCV-RNA assays as a criterion of active infection, FDA is not presuming any indications for use for HCV-RNA assays that might be considered via the PMA process.

(2) Acute infection

should be demonstrated by testing multiple specimens from the same individual: comparative assays should not detect anti-HCV in earlier specimens and should detect anti-HCV in later specimens. For determining performance with regard to indications that pertain to acute HCV infection (Indications 3 and 5: IV.C above and V.C below), FDA recommends testing of at least four specimens (i.e., two successive specimens yielding negative results via comparative anti-HCV testing, followed by two successive specimens yielding positive results; with current assays, the mean time from exposure to seroconversion is 8-9 weeks {ref. 3}). If only one anti-HCV-negative specimen is available from certain individuals, other temporal characteristics could establish that the individual was acutely infected at the time subsequent specimens were collected. For determining performance of new assays that are thought to be more sensitive than older assays, FDA recommends testing of serially collected blood for HCV RNA to demonstrate onset of HCV replication.

(3) Chronic infection

should be demonstrated by testing two or more specimens collected from the same individual during an interval of at least 6 months: comparative assays should detect anti-HCV for ≥ 6 months.

(4) Genotype

is an important, but controversial, consideration because some, but not all, investigators have presented evidence that certain HCV genotypes and subtypes are associated with more severe disease and with decreased response to antivirals. (Serotypes, as classically defined by antigenic cross-reactivity and protection within a serotype, have not yet been identified for HCV. On the basis of sequence diversity alone, it is quite possible that the HCVs represent two or more distinct viral species.) Furthermore, there are no FDA-approved assays for identifying HCV genotypes and performance varies widely among commercially available and research assays {ref. 4}. There has also been controversy about consensus definitions for and the number of recognized HCV genotypes. FDA believes, however, that PMAs should include as much information as possible about infecting genotypes because recognized genotype-dependent differences in performance of non-approved assays for anti-HCV or HCV RNA have led to false results or quantitative errors that are likely to have adverse effect on patient management. The manufacturer should apply the considerations in section V.6.b.(1) and in reference {ref. 4} for developing information about infecting HCV genotypes among certain patients or specimens.

c) Inactive HCV infection

should be demonstrated by comparative-assay testing of three or more specimens from each individual:

(1) In a group not treated with anti-HCV therapy

(2) Following treatment, in a group having "sustained virologic and biochemical responses" (i.e., undetectable HCV viremia and within-reference-range ALT concentrations) during an interval of at least 1 year: HCV RNA should not be detectable in all tested specimens for ≥ 12 months. In addition, the individual should not have evidence of hepatitis (V.B.6.a.) during the sampled interval.

d) Different types of populations should be studied for determining specificity and for estimating prevalence ("Expected Values") as detected by the manufacturer's new assay:

(1) Patients who have hepatitis or other HCV-associated syndromes (e.g., porphyria cutanea tarda), without evidence of HCV infection are appropriate for studying clinical specificity (for examples, see Table 3 and Figure 1).

(2) Healthy individuals are appropriate for studying specificity with regard to indications that pertain to asymptomatic HCV infection (Indications 3 and 4: IV.C above and V.C below) and for determining prevalence. Although blood donors are often used for such studies, it is important to recognize that they represent selected populations: even first-time donors are screened via questionnaire before specimens are collected. Repeat donors have been screened via lab testing during previous donations. The best estimates of asymptomatic specificity and prevalence would be determined from healthy members of populations that were demographically similar to those being studied for Indications for Use.

To interpret a prevalence study for a new anti-HCV assay, results should be presented as % new-assay-positive (% new-assay negative would be an estimate of minimum specificity among asymptomatic individuals). Comparative testing results should not be used to interpret the specificity of new-assay results, unless comparative testing has been applied to statistically appropriate subsets of specimens that yielded positive and negative results with the new assay.

7. Inclusion and exclusion criteria for specimens

should include conditions for collection, handling, and storage. Protocols should indicate how these criteria will be met and documented. An explanation should be provided for each group of specimens that will be studied, especially if they represent an archive of frozen specimens or a group of individuals for whom individual clinical characteristics will not be provided. Specimen archives, including "panels" from commercial suppliers, should be described in terms of criteria and introduced biases for inclusion in the archive, number of individuals represented (e.g., each "seroconversion panel" should represent only one individual), criteria and introduced biases for selecting certain specimens to study, and how the archive has been stored (including criteria for and documentation of monitoring during storage).

8. Laboratory procedures – new assay

a) Specimens should be masked

i.e., personnel who perform the studies and interpret the data should be blinded with regard to any characteristics about each specimen, including results from comparative and other assays.

b) Quality control

procedures should be performed, for each run, according to the assay's instructions for use in the clinical study protocol. These instructions should be identical to those in a draft package insert. In the PMA, line data should include lot number and quality control results for each run during clinical studies, including documentation of failed runs.

9. Data analysis

a) Analysis should be masked

i.e., personnel who assign individuals or specimens into categories should be blinded with regard to new-assay results.

b) Performance characteristics.

The manufacturer should provide a statistically based value for variability among calculated performance estimates. For example, sensitivity and specificity figures typically include 95% confidence intervals.

(1) For characteristics that pertain to qualitative diagnostic indications, performance should be expressed in terms of % new-assay results that are "correct," where correct refers to the category to which individuals or specimens have been assigned, according to criteria in the clinical protocol. For examples, please refer to Table 2 and Figure 1.

It is appropriate to describe a particular performance characteristic for an unmatched population (e.g., "specificity among first-time blood donors" or "clinical specificity among patients with acute hepatitis A"). Similarly, it might be difficult to determine the state of infection (acute versus chronic) in a population of patients with hepatitis but who are not HCV-infected. It would be appropriate, for example, to calculate "clinical specificity among patients with acute or chronic hepatitis" and "clinical sensitivity among patients with chronic hepatitis C." Such findings should be presented separately because they represent different populations.

(2) Performance for diagnostic indications with qualitative assays should also include validation of cutoff(s). The manufacturer should present data to demonstrate that each cutoff is appropriate, as determined from clinical studies of well-characterized individuals or specimens. Such presentation typically includes a graphic representation of data, in such forms as a ROC curve or a histogram (number of new-assay results versus new-assay values,

with the cutoff marked on the horizontal axis). It is not appropriate to *validate* a cutoff by using results from two different populations (e.g., positive results primarily from patients with hepatitis C and negative results primarily from blood donors).

(3) For characteristics that pertain to temporally variable data, such as seroconversion during acute HCV infection (diagnosis) or HCV-RNA concentrations at any time during infection (monitoring), the manufacturer should analyze and present data by using time as an independent variable. For example, results for a qualitative assay for anti-HCV could be presented in tabular form as % new-assay-positive (with 95% confidence intervals) during intervals after exposure or before hepatitis. Results for a quantified HCV-RNA assay could be presented in graphic form, as concentration versus time, with error bars for each data point.

c) "Discrepancy resolution"

Studies will not have "discrepant" results because each individual or specimen should be categorized according to study criteria that may include comparative-assay and other laboratory results. If the new assay being studied is an EIA or SIA for anti-HCV, data from additional testing with comparative assays may be useful for a better understanding of the nature of false-positive reactivity.

It is not, however, appropriate to "resolve" certain false-positive results to a "true positive" interpretation on the basis of additional-testing data, unless study protocols included plans for additional testing of a statistically determined subset of specimens that initially yielded a true-negative interpretation. If so, new-assay-negative / additional-testing-positive results should then be interpreted as false-negative. That is, all additional-testing-positive results would lead to categorization as "HCV infection, state of infection or associated disease not determined," regardless of new-assay results.

C. Design and Protocols: Additional Recommendations for Clinical Studies to Determine Performance for Specific Indications for Use

1. Evidence of HCV infection, not specified with regard to state of infection or associated disease

General considerations for specimens (please refer to section V.B above, "Design and Protocols for Clinical Studies: General Considerations") should be applied to studying this indication. For a suggested testing and analysis algorithm, please see Table 2.

Table 2. Data analysis: supporting claim for "evidence of HCV infection, not specified with regard to state of infection or associated disease" (Indication 1 per sections IV.C and V.C)

A. New assay for presumptive (1st-step) or stand-alone (only-step) detection of anti-HCV (e.g., EIA)

Category per Table 1	Testing with new assay		Performance	
	Result	**Interpretation**	**Calculate**	**Characteristic [a]**
Not HCV-infected	Negative	TN	TN / (TN + FP)	"Specificity for individuals without serologic evidence of active or inactive HCV infection"
	Positive	FP		
Anti-HCV seroconversion	Negative	TN		
HCV infection, state or associated disease not determined	Negative	FN	TP / (TP + FN)	***New presumptive assay*** "Sensitivity for detecting presumptive serologic evidence of HCV infection (not specified with regard to state of infection or associated disease); positive results should be supplemented by testing with another assay that is more specific for HCV infection" ***New stand-alone assay*** "Sensitivity for detecting serologic evidence of HCV infection (not specified with regard to state of infection or associated disease)"
	Positive	TP		
Anti-HCV seroconversion	Positive	TP		

[a] These performance characteristics should not be referred to as "clinical" sensitivity or specificity nor should the manufacturer calculate predictive values, because *evidence of HCV infection, not specified with regard to state of infection or associated disease* is not a clinical indication for use. If these characteristics were determined in different populations, they should be separately displayed in the draft package insert.

B. New assay for supplemental (2nd-step) detection of anti-HCV (e.g., SIA)

Category per Table 1	Testing with new assay		Performance	
	Result	**Interpretation**	**Calculate**	**Characteristic**
Not HCV-infected Anti-HCV seroconversion	Do not test	(None)	(None)	(2^{nd} step testing not indicated for specimens that yield Negative for 1^{st}-step. To determine "Expected Values," perform new-assay testing on unselected populations)
HCV infection, state or associated disease not determined	Negative Positive	FN TP	TP / (TP + FN)	"Sensitivity for detecting supplemental serologic evidence of HCV infection (not specified with regard to state of infection or associated disease)" [b]
Anti-HCV seroconversion	Positive	TP		

[b] This performance characteristic should not be referred to as "clinical" sensitivity because *evidence of HCV infection, not specified with regard to state of infection or associated disease* is not a clinical indication for use.

If additional patient information for certain specimens will be available, it should be indicated in protocols and provided in the PMA. Even though there may not be enough data to support other indications for use, performance for other indications might be suggested by these data (for examples, please see Table 3).

Table 3. Indication 1 ("evidence of HCV infection, not specified with regard to state of infection or associated disease"): examples of groups and subgroups that suggest, but do not demonstrate, performance with regard to states of infection or associated disease[a]

A. "Not HCV-infected" category: specificity suggested

Group	Subgroup	Per criteria	Calculate "% [New assay] Positive among
Hepatitis [b]		Present	...patients with hepatitis"
		Absent	...individuals without hepatitis"
Hepatitis present	Symptoms of hepatitis	Present	...patients with symptomatic hepatitis"
		Absent	...asymptomatic individuals with hepatitis"
	Acute HAV infection [b]	Present	...patients with hepatitis A"
	Active HBV infection [b]	Present	…patients with acute or chronic HBV"
	Active HDV infection	Present	…patients with acute or chronic HDV"
	Acute CMV infection	Present	...acute CMV-associated hepatitis"
	Acute EBV infection	Present	...acute EBV-associated hepatitis"
AIDS		Present	...patients with AIDS"

B. "HCV infection, state of infection or associated disease not determined" category: sensitivity suggested

Group	Subgroup	Per criteria	Calculate "% [New assay] Positive among
Hepatitis [b]		Present	…patients with hepatitis"
		Absent	...individuals without hepatitis"
Hepatitis present	Symptoms of hepatitis	Present	...patients with symptomatic hepatitis"
		Absent	...asymptomatic individuals with hepatitis"
	Active HBV infection [b]	Present	…patients with concurrent hepatitis B"
	AIDS	Present	…patients with AIDS and hepatitis"
State of infection [c]		Active	...individuals with active HCV infection"
		Inactive	...individuals with inactive (no evidence of current replication) HCV infection"
Active infection	Acute	Present	...acutely HCV-infected individuals"
	Chronic	Present	...chronically HCV-infected individuals"
Infecting genotype known		Determined	...individuals infected with HCV genotype [specify]"

[a] These lists represent examples of optional (i.e., they go beyond the minimum recommendations for Indication 1) groups and subgroups that are intended to provide ideas for appropriate types of data and data analysis but are not intended to be all-inclusive. Alternative analyses could be performed by having a different hierarchy of groups and subgroups.

[b] Physical signs, biochemical evidence, or histopathologic evidence per section V.B.6.a on criteria for hepatitis; microbiologic evidence of infection with an agent that causes hepatitis (e.g., acute HAV infection ≡ positive for anti-HAV IgM, active HBV infection ≡ HBsAg-positive)

[c] Inactive ≡ no evidence of hepatitis, repeatedly negative for HCV RNA, and may represent resolved infection; Active ≡ HCV-RNA Pos or evidence of recent seroconversion (please refer to section V.B.6 above, on inclusion and exclusion criteria).

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2. Screening or managing donors of blood, plasma, tissue, or organs

Please contact CBER for recommendations on studies to support a license application for this indication.

3. Aid in detecting acute asymptomatic HCV infection

Please refer to General Considerations above in sections V.B.6.b.(1) and (2); in addition,

a) Population should represent individuals at high risk of HCV exposure
b) Case report forms should be considered, with entries for data to demonstrate absence of symptoms and biochemical abnormalities
c) Serial specimens from individuals should be tested to establish if and when the new EIA yields positive results. Please see section V.B.6.b.(2) above, on acute infection.

4. Aid in detecting chronic asymptomatic HCV infection

Please refer to section V.B.6.b.(3) above, on chronic infection, and to V.C.3.a) and b) immediately above, on population and case report forms. The new assay should not be studied with specimens from the acute phase of infection.

For these "asymptomatic" Indications 3 and 4, specificity could also be estimated in a population of "healthy" individuals by categorizing according to Table 1 (please also refer to V.B). This performance characteristic should be expressed separately from those for an at-risk population; please see section V.B.9.b.(1) above, on data analysis for performance characteristics.

5. Aid in diagnosis of acute hepatitis C

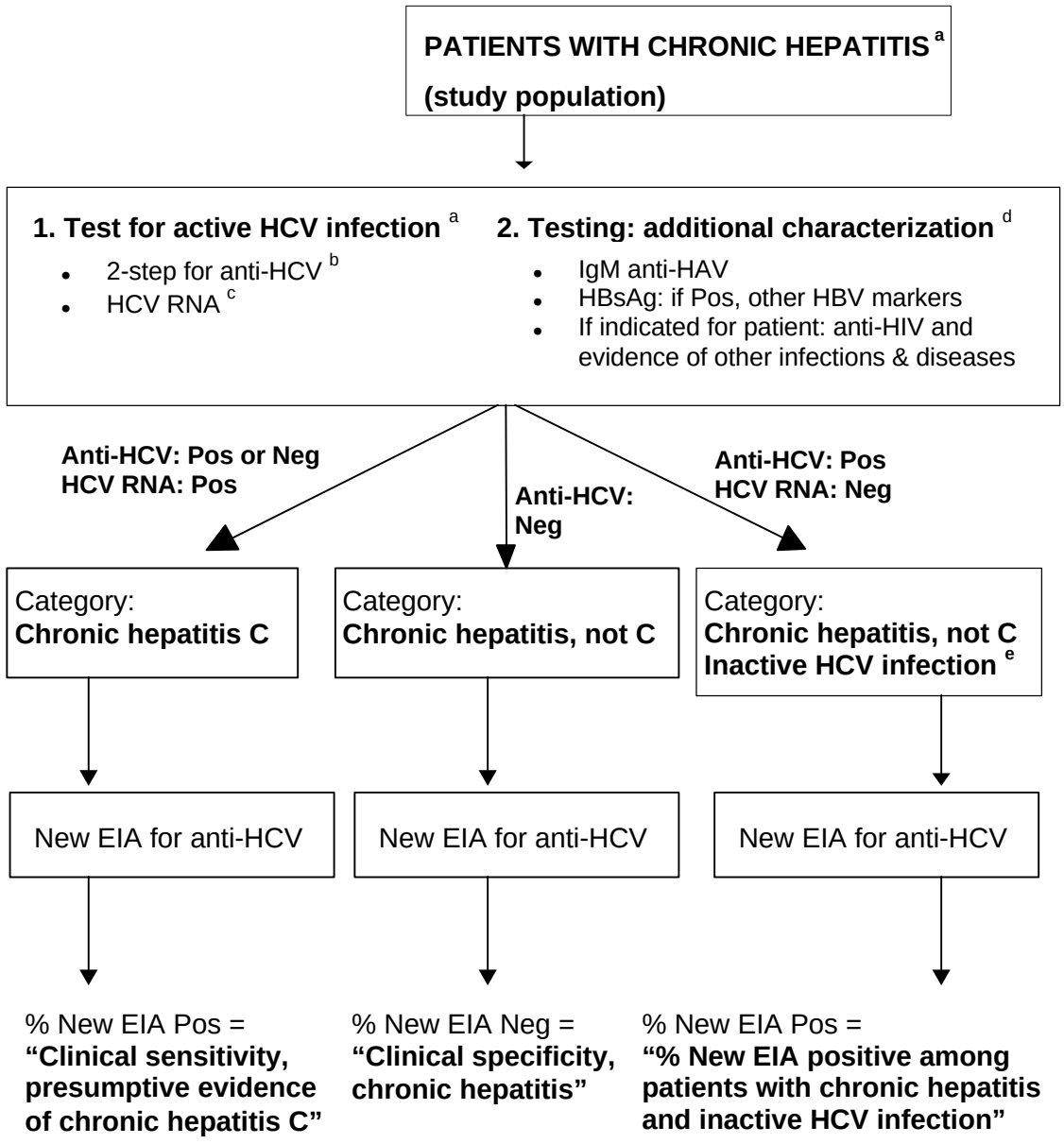
Please refer to sections V.B.6.a and B.6.b.(1) & (2) above, on inclusion and exclusion criteria, and section V.C.3.c) immediately above, on serial specimens. In addition, case report forms should be considered, with entries for data to demonstrate symptoms, biochemical abnormalities, and other evidence of hepatitis; please refer to section V.B.6 above, on inclusion and exclusion criteria.

6. Aid in diagnosis of chronic hepatitis C

Please refer above to sections V.B.6.a and b.(3) above, on criteria for chronic hepatitis and to section V.C.5 immediately above, on case report forms. The new

assay should not be studied with specimens from the acute phase of infection. See Figure 1 for a suggested algorithm.

Figure 1. Determining performance: new EIA as aid for presumptive diagnosis of chronic hepatitis C



[a] Inclusion criteria for "hepatitis" and "chronic": please refer to V.B.6.a. and V.B.6.b.(3).
[b] "2-step testing" for anti-HCV: please refer to V.B.4.a. and Table 1.
[c] Criteria for unapproved HCV-RNA assays: please refer to V.B.6.b.(1).
[d] Additional characterization may enable sub-categorization and additional conclusions about new EIA performance; please refer to V.B.4.b.
[e] Definition of "inactive HCV infection": please refer to V.B.5.c.

7. Aiding diagnosis of hepatitis C (indiscriminate between acute and chronic)

This indication should be supported by more data than Indication 1 (i.e., evidence of hepatitis and of active HCV infection: please refer to sections V.B.6.a and b above, on inclusion and exclusion criteria) but less than Indications 5 or 6 because temporal data would not be necessary.

8. Monitoring active HCV infection

a. Prognosis of acute HCV infection

b. Prognosis of chronic HCV infection without antiviral therapy

c. Predicting response of chronic HCV infection to antiviral therapy

d. Monitoring response of chronic HCV infection to antiviral therapy

e. Prognosis of chronic HCV infection after antiviral therapy is completed or discontinued

Several of these indications are already important uses for HCV-RNA assays. For each, several considerations apply:

- The manufacturer should determine HCV-RNA concentrations, per the new assay, that correspond to clinical-decision points. When the new assay is qualitative, this consideration pertains to selection of one or more cutoffs.
- The manufacturer of a new quantitative assay should determine values that correspond to clinically significant change(s) in HCV RNA concentration.
- Endpoints should be selected for studies:

  Results of comparative HCV-RNA assays should be considered while recognizing that none are FDA-approved; please refer to section V.B.6.b.(1) above, on detection of HCV RNA.

  Criteria for clinical improvement or worsening, which could include symptoms, signs, ALT and other biochemical markers, and quantified histopathologic changes. Please refer to section V.B.6.a above, on criteria for hepatitis.

  Length of study period, premarket or postmarket – the manufacturer should consider if the new assay's utility pertains to short terms (months to a few years) or for longer periods during which the most serious complications of HCV infection may develop.

FDA recognizes that these indications represent a new and rapidly changing area of medical research and practice. For some indications, it may not yet be possible to design appropriate studies. For others, the interval between specimen collection and serious complications of HCV infection may be so long that effects of assay result on outcome cannot be practically studied. Until proven otherwise, FDA will assume that a decrease in HCV-RNA concentration does not represent a detrimental event (where "decrease" should be based on preclinical parameters that include precision, interference, specimen handling, reagent stability, and effective calibration and quality control.)

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Note: all US government documents that are published in Federal Register *or in* CFR *can be retrieved from the Internet at* http://www.access.gpo.gov/su_docs/aces/aces140.html

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